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IN-VIVO AND *IN-VITRO* MAST CELL STABILIZING ACTIVITY OF ETHYL ACETATE AND METHANOL EXTRACT OF *TERMINALIA CHEBULA* FRUITS: A THERAPEUTIC APPROACH FOR ASTHMA

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ABSTRACT: The present investigation deals with the *in-vivo* and *in-vitro* mast cell stabilizing the activity of fruits of *Terminalia chebula* ethyl acetate and methanol extracts at 500 mg/kg body weight. In egg albumin induced degranulation studies, Kitotifen as a standard was found to inhibit degranulation to an extent of 80.12%, where as ethyl acetate and methanolic extracts inhibited degranulation to 58.98% and 44.56% respectively similarly in compound 48/80 induced mast cell degranulation in rats ethyl acetate and methanol extracts of *Terminalia chebula* and standard Kitotifen showed the following percentage inhibition of degranulation of mast cell 40.28%, 51.19% and 75.12 respectively. In *in-vivo* study ethyl acetate and methanolic extracts at the dose of 500 mg/kg bodyweight inhibited degranulation of mast cell to the extent of 46.5 % and 37.33% respectively.

INTRODUCTION: Asthma may be defined as a condition with recurrent 'reversible' obstruction of the airflow in the airways in response to stimuli which are not in themselves noxious and which do not affect non-asthmatic subjects [1]. Asthma is a major global public health problem. The prevalence, and perhaps also severity of the disease is increasing, in particular in urbanized areas around the world. In many countries, the prevalence of asthma is around 5% in the adult population and 10% or higher among children below the age of 10. Although the genetic and environmental causes of asthma remain to be defined more precisely, the recognition of asthma as a chronic inflammatory disorder of the airway has greatly focused etiological and therapeutic research.

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Bronchial Asthma according to the GINA guidelines final update November 2006 is clearly defined as: "A chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyper responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing, particularly at night or in the early morning." These episodes are associated with airflow obstruction within the lung that is often reversible either spontaneously or with treatment [2, 3]. Mast cells are constituents of virtually all organs and tissues and are important mediators of inflammatory responses such as allergy and anaphylaxis.

In which histamine remains the best characterized and most potent vasoactive mediator implicated in the acute phase of immediate hypersensitivity upon release [4]. Mast cells are broadly distributed throughout mammalian tissues and play a critical role in a wide variety of biologic responses. Typically, mast cells have been considered not only in the association of immediate-type hyper-

sensitivity but also in late reactions, like inflammatory responses [5]. Immediate-type hypersensitivity is mediated by histamine released in response to the antigen cross-linking of immunoglobulin E (IgE) bound to FceRI on the mast cells [6]. After activation via the FceRI, the mast cells start the process of degranulation, which results in the releasing of mediators, such as products of arachidonic acid metabolism and an array of inflammatory cytokines [7].

Among the inflammatory substances released from the mast cells, histamine is one of the best characterized and most potent vasoactive mediators implicated in the acute phase of immediate hypersensitivity [8]. The morbidity and mortality of asthma have increased over the past two decades, particularly in Western countries [9]. In most cases, mild-to-moderate asthma is controlled by inhalational steroid. However, long-term steroid therapy is often associated with adverse effects [10]. Many side effects of steroids including adrenal suppression and reduction in growth velocity have been reported [11]. There is a need for the development of additional effective treatments with fewer side effects.

Fruits of *Terminalia chebula* Retzius (*T. chebula* Retz.) (Combretaceae), commonly known as black Myrobalans in English and Harad in Hindi, indigenous in Pakistan and India among many Asian and African countries, is a popular folk medicine and has been studied for its homeostatic, antitussive, laxative, diuretic and cardiotonic activities [12, 13]. *Terminalia chebula* is routinely used as traditional medicine by tribals of Tamil Nadu to cure several ailments such as fever, cough, diarrhea, gastroenteritis, skin diseases, candidiasis, urinary tract infection and wound infections [14]. Phytochemical investigations of *Terminalia Chebula* have been reported on the presence of tannins, carbohydrates, glycosides, phenols, alkaloids, terpenoids and flavonoids [15].

MATERIALS AND METHODS:

Collection of Plant Material: The fruits of *Terminalia chebula* was collected from the local market, Indore. The plant material was identified at Department of Botany, Holkar Science College, Indore, and their voucher specimens were deposited in the Department of Pharmacognosy, School of Pharmacy, Devi Ahilya Vishwavidhyalaya, Indore.

Preparation of Plant Extracts: The air-dried plant material was reduced to coarse powder and subjected to successive solvent extraction with solvents Pet. Ether, Chloroform, Ethyl acetate and Methanol in Soxhlet extractor. After the complete extraction, the solvent was distilled off and concentrated on a water bath to a dry residue. The extracts were concentrated by distilling off the solvent and then evaporated to dryness on a water bath. Ethyl acetate and the methanolic group showed the presence of maximum phytochemical constituents that's why only these two groups for undertaken for further study [16, 17].

Animals: Animals-Male Wister rats (200-250g) were obtained from the experimental animal house. The animals were housed in polypropylene cages under standard conditions (12 h light; 12 h dark cycle; 25± 5 ºC; 35-60% humidity). They were fed with standard pellet diet (Pranav Agro Ltd, Dehradun) and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC/PCP/2014/49).

Evaluation of *in-vitro* Mast Cell Stabilizing Activity: Sensitized mast cell was obtained from animals sensitized with egg albumin. The doses to the animal groups (Six animal in each group); Group 1: Control, Group 2: Kitofen (Standard), Group 3: Ethyl acetate extract of *Terminalia chebula*, 500 mg/kg body weight; Group 4: methanolic extract of *Terminalia chebula* 500 mg/kg body weight being given on the 1st, 3rd and 5th day. The sensitized mast cells were degranulated using egg albumin (1mg/ml) on the 10th day of sensitization. The normal mast cell was degranulated using compound 48/80 (100mcg/ml). To the cell suspension of mast cells 0.1 ml of the peritoneal mast cell suspension, 0.1ml of the test agent in the saline was added and incubated in a constant temperature water bath (37 ºC) for 15 min. Then 0.1 ml of degranulating agent (Egg albumin 1 mg/ml and compound 48/80 100mcg/ml) was added and further incubated for 10 minutes. The cell was then stained with 0.1% toluidine blue for 5-10 min, and the tissue was then washed in acetone and then xylene (2 changes each) for 5 min each wash.

The stained cells were viewed through a digital light microscope at 100x magnification, and 100 mast cells were counted. The number of intact and fragmented or disrupted mast cells was noted.

A mast cell was considered disrupted if four or five granules were found around the mast cells. The number of fragmented or disrupted mast cells as well as of the intact mast cells were counted [18].

Group 1: Control, Group 2: Kitofen (Standard), Group 3: Ethyl acetate extract of *Terminalia chebula*, 500 mg/kg body weight; Group 4: methanolic extract of *Terminalia chebula* 500 mg/kg body weight) rats were sensitized by injecting subcutaneously 0.5 ml of horse serum along with 0.5 ml triple antigen containing 20,000 million *Bordetella pertussis* organism. Rats of group 1 received water (vehicle) and served as control Rats of group 3 and 4 were administered herbal extracts p.o. respectively, once a day for 14 days. Group 2 rats received 10 mg/kg of Prednisolone (standard) orally for the same duration. On day 14, the rats were sacrificed two hours after treatment, and the intestinal mesentery was taken for the study on mast cells. Mesenteries of sacrificed rats along intestinal pieces were kept in Ringer –Locke solution.

The mesenteric pieces were challenged with horse serum for 10 min after which the mast cell was stained and examined microscopically for the number of the intact and degranulated mast cell. Pieces of intestinal mesentery will be mounted on a slide which will be air dried and then stained with 1% toluidine blue, at room temperature for 5 min. Mast cell will be readily identified by their metachromatic cytoplasmic granules under light microscope [19].

RESULT: All experimental data were expressed as mean ± SEM. Table Statistical analysis was carried out by using one way ANOVA followed by Dunnett's test. In the present study, the rats of unsensitized group, *i.e.* control group showed 12.33 ± 1.03% of degranulated mast cell whereas standard drug Prednisone found to inhibit degranulation of mast cell by 30.16 ± 2.13%. The ethyl acetate and methanolic extracts at the dose of 500mg/kg bodyweight inhibited degranulation of mast cell to an extent 46.5 ± 2.16% and 37.33 ± 2.73% respectively.

TABLE 1: *IN-VIVO* MAST CELL STABILIZING ACTIVITY OF *TERMINALIA CHEBULA* EXTRACT

Treatment	Doses (mg/kg body weight)	Route of Administration	Granulated mast cell	Non -Granulated mast cell
Control (TWEEN 80, 1)*		Oral	85.83 ± 1.72	12.33 ± 1.03
Control (TWEEN 80, 1) sensitize		Oral	23.66 ± 3.32	82.5 ± 3.06
Prednisolone	10	Oral	75.66 ± 2.42	30.16 ± 2.13
Ethyl acetate *Terminalia chebula*	500	Oral	68.83 ± 1.72	46.5 ± 2.16
Methanolic *Terminalia chebula*	500	Oral	65.33 ± 2.33	37.33 ± 2.73

*: $p<0.05$ *vs*. control n= number of animals

TABLE 2: EFFECT OF *TERMINALIA CHEBULA* EXTRACTS ON EGG ALBUMIN INDUCED MAST CELL DEGRANULATION IN RATS

Treatment N=6	Dose (mg/kg)	Number of mast cells	Percent inhibition
Control		9 ± 1.3	
Ketotifen	10 mcg/ml	88 ± 1.5 *	80.12 ± 1.02
Ethyl acetate extract of *Terminalia chebula*	500	60 ± 1.03 *	58.98 ± 1.04
Methanol. Extract of *Terminalia chebula*	500	46 ± 0.17 *	44.56 ± 1.01

*: $p<0.05$ *vs*. control n= number of animals

TABLE 3: EFFECT OF *TERMINALIA CHEBULA* EXTRACTS ON COMPOUND 48/80 INDUCED MAST CELL DEGRANULATION IN RATS

Treatment N=6	Dose (mg/Kg)	Number of mast cells	Percent inhibition
Control		6 ± 0.11	
Ketotifen	10 mcg / ml	78 ± 1.26*	75.12 ± 1.08
Ethyl acetate extract of *Terminalia chebula*	500	52 ± 1.13*	51.19 ± 1.27
Methanol. Extract of *Terminalia chebula*	500	41 ± 0.13*	40.22 ± 1.45

*: $p< 0.05$ *vs*. control n= number of animals

DISCUSSION: Present investigation aimed to evaluate the asthamatic activity by mast cell stabilizers model. In mast cell stabilizing study, the efficacy of the drug to prevent mast cell degranulation is observed. Upon injecting allergen horse serum to the test animals, IgE production is enhanced leading to an increase in histamine. This leads to an increase in Inositol triphosphate leading to calcium ion influx. This causes degranulation of mast cells. The control group animals showed maximum mast cell degranulation as observed from histopathology slides. Upon administration of extracts, the degranulation was decreased. This might be due to mast cell stabilizing potential against antigen-antibody reaction or due to suppression of IgE antibody production, responsible for degranulation [20].

Mast cells are known to be the primary responders in allergic reactions, most of which are triggered by cross-linking of a high-affinity IgE receptor (FC RI). Allergic manifestations include allergic rhinitis, anaphylaxis, purities, and asthma - the diseases associated with inflammatory conditions. In mast cell stabilizing study, the efficacy of the drug to prevent mast cell degranulation was observed [21].

Methanolic and ethyl acetate extracts of *Terminalia chebula* markedly protected the sensitized mast cells. However, the effect was less than that observed with the standard drug used. The pathological mechanism involved in Type-I allergy has been explained as the degranulation of mast cells and basophils, followed by the release of mediators such as histamine, leukotrienes, and prostaglandins from these cells.

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